

Phytoremediation Using Microbial Communities: II

Elizabeth Temitope Alori

15

E. T. Alori , M.Sc., Ph.D. (*)
Department of Crop and Soil Science , Landmark University ,
Omu Aran , Kwara State , Nigeria
e-mail: aloritope@yahoo.com
aloritope@yahoo.com

15.1 Introduction

Phytoremediation is a novel green technology that uses specialized plants and associated soil microbes to remove, destroy, sequester or reduce the concentrations or toxic effects of contaminant in polluted environment especially soil and water. It refers to a group of plant-based technologies that use either naturally occurring or genetically engineered plants to clean contaminated environments. This technology depends on the ability of both the plant and associated microorganisms to adapt to or survive in high-metal environments. Polluted soil poses a severe problem to both ecosystem health and land development. Soil pollution threatens the health of human, plant and animal. Soil pollution can spread to other parts of the natural environment because soil is at the confluence of many natural systems. For instance, groundwater that percolates through a polluted soil can carry soil contaminants into streams, rivers, wells and drinking water. Plants growing on polluted soil may contain harmful levels of pollutants that can be passed on to the animals and people that eat them. Dust blown from polluted soil can be inhaled directly by passers-by. Additionally, polluted soil renders valuable open land unusable for parks, recreation or commercial development. The fact that both soil minerals and soil pollutants carry small electric charges that cause each to bond with each other makes polluted soil very hard to clean. A range of technologies such as fixation, leaching, soil excavation, chemical treatment, vitrification, electrokinetics and landfill of the top contaminated soil, bioventing, thermal desorption, soil vapour extraction, biopiles, etc., have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution (Haque et al. 2008). Excavation of polluted soil for off-site treatment or disposal is labour intensive, consumes a lot of time and requires the use of heavy machinery hence very expensive (Danh et al. 2009). Therefore, cheaper on-site, or in situ, remediation techniques have recently become the focus of research. One of the most interesting and promising of these in situ techniques is phytoremediation. Using plants to remediate soil pollution comprised of two components, one by the root-colonizing microbes and the other by plants themselves which absorb, accumulate, translocate, sequester and detoxify toxic compounds to non-toxic metabolites. Plants frequently lack metabolic capacity for the degradation of many pollutants hence the need to utilize degradation ability of soil organisms. Metal tolerance of plants is generally increased by symbiotic, root-colonizing, arbuscular mycorrhizal fungi (AMF), through metal sequestration in the AMF

hyphae. More also excretion of the glycoprotein glomalin by AMF hyphae can form complex metals in the soil. Exposure of plants to microorganisms within the rhizosphere protects the plants from the toxic effect of the contaminants and also takes part in phytoremediation. Resistant plants can thrive on sites that are too toxic for other plants to grow. They in turn give the microbial processes the boost they need to remove organic pollution more quickly from the soil.

The mechanism responsible for the phytoremediation of contaminated soil has been proved to be as a result of increase in microbial activity. Organic toxins, those that contain carbon such as the hydrocarbons found in gasoline and other fuels, can be broken down by microbial processes.

Soil fungi, for example, improve phytoremediation ability of plants by increasing the absorptive area of the roots of plants. The efficiency of *Tithonia diversifolia* and *Helianthus annuus* in remediating soils contaminated with zinc and lead nitrates could be improved by introducing mycorrhizal fungi in order to increase the absorptive area of the roots of these plants (Adesodun et al. 2010). Plants on the other hand play a key role in determining the size and health of soil microbial populations. All plant roots secrete organic materials that can be used as food for microbes, and this creates a healthier, larger, more diverse and active

184

microbial population, which in turn causes a faster breakdown of pollutants. Phytoremediation reduces contaminant levels through microbial degradation in the rhizosphere. Phytoremediation systems increase the catabolic potential

of rhizosphere soil by altering the functional composition of the microbial community (Siciliano et al. 2003). Plants, through their “rhizosphere effects”, support hydrocarbon-degrading microbes that assist in phytoremediation in the root zone (Nie et al. 2009). For example, root activities in perennial ryegrass and alfalfa increase the number of rhizobacteria capable of petroleum degradation in the soil

(Kirk et al. 2005). In turn, healthy microbial communities enhance soil nutrient availability to the plants (Wenzel

2009). Phytoremediation process can also be enhanced by the addition of specific inocula of microorganism to contaminated soils (bioaugmentation). Also, plants that are relatively

tolerant to various environmental contaminants are often stunted in the presence of the contaminant. Therefore, plant growth-promoting microorganisms can be added to the roots of plants to remedy this situation.

The best bioaugmentation performance can be achieved by the use of microorganisms that are already present in the soil, since indigenous microorganisms are well adjusted to their own

environment. Inoculating plants with genetically engineered strains of bacteria that degrade a specific contaminant has

shown promising results. Biostimulation, a process which involves manipulating the nutrient and pH levels of the soil to increase microbial populations, can also be used to amplify the population of soil organism responsible for biodegradation.

Hence, fertilizers can be used together with bioaugmentation to facilitate degradation of pollutants.

15.2 Advantages of Phytoremediation

Using Microbes

1. Low-cost: It is less expensive than alternative engineering-based solutions such as soil excavation, incineration or landfilling of the contaminated materials.
2. Aesthetically pleasing and appealing to the public. Trees

and smaller plants used in phytoremediation make a site more attractive, reduce noise and improve surrounding air quality.

3. Site use and remediation can occur simultaneously.
4. In situ approach: It treats the contamination in place so that large quantities of soil, sediment or water do not have to be dug up or pumped out of the ground for treatment.
5. Environmentally friendly: Poses no health risk to neither plant, human nor animal.
6. Enhance soil nutrient availability to the plants.
7. It takes advantage of natural plant processes and requires less equipment and labour than other methods since plants do most of the work.
8. Saves energy since the site can be cleaned up without digging up and hauling soil or pumping groundwater.
9. Trees and smaller plants used in phytoremediation help control soil erosion.
10. Creates a more fertile soil as soil organic matter is increased as a result of root secretions and falling stems and leaves.
11. Phytoremediation does not degrade the physical or chemical health of the soil as compared to soil excavation method that removes the organic-matter-rich topsoil and, because of the use of heavy machinery, compacts the soil that is left behind.
12. Its by-product can find a range of other uses. Some of the plants used for phytoremediation produce metabolites or phenolic compounds that are of commercial value in the pharmaceutical industry.
13. The roots of plants used create pores through which water and oxygen can flow.

15.3 Limitations of Phytoremediation

Using Microbes

1. A long time period is required for remediation. It is a slow process that may take many growing seasons before an adequate reduction of pollution is achieved, whereas soil excavation and treatment clean up the site quickly. Multiple metal-contaminated soils require specific metal accumulator species and therefore require a wide range of research prior to the application. The cadmium/zinc model hyperaccumulator *Thlaspi caerulescens*, for example, is sensitive towards copper (Cu) toxicity, which is a problem in remediation of Cd/Zn from soils in the presence of Cu by application of this species.
2. Scientific understanding of mechanisms is still limited; this is because the technique is still in its infancy state.
3. Hyperaccumulators can be a pollution hazard themselves. For instance, animals can eat the hyperaccumulators and cause the toxins to enter the food chain. If the concentration of contaminant in the plants is high enough to cause toxicity, there must be a way to segregate the plants from humans and wildlife, which may not be an easy task.

15.4 Environmental Contaminants

The following compounds have been reported as contaminants in soil and water:

Pesticides; explosives; oil; heavy metal such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), uranium (U), vanadium (V) and wolfram (W); polychlorinated biphenyls; polycyclic aromatic hydrocarbons (PAHs); chlorinated solvents; xenobiotics;

munitions; semi-coke solid wastes (which contain several

E.T. Alori

aloritope@yahoo.com

185

organic and inorganic compounds such as oil products, asphaltenes, phenols, PAHs, sulphuric compounds); oil shale; and organic synthetic compounds.

15.5 Factors that Affect Phytoremediation

Certain factors affect the uptake, distribution and transformation of contaminants. Some of these factors include the following:

1. Level of contamination: They work best where contaminant levels are low because high concentrations may limit plant growth and take too long to clean up.
2. Plant species used for phytoremediation: Certain plants are better at removing contaminants than others. This may be due to differences in root exudate patterns, differences in root architecture as well as differences in genetic composition of the plant. Tall fescue with fibrous root system, for example, increases the potential of soil microbial community to degrade hydrocarbons, whereas rose clover with a coarse, woody root system decreases it (Siciliano et al. 2003). Plants used for phytoremediation must be able to tolerate the types and concentrations of contaminants present. They also must be able to grow and survive in the local climate. Chemical, physical and microbiological plants with low biomass yield and reduced root systems do not support efficient phytoremediation and most likely do not prevent the leaching of contaminants into the aquatic system.
3. Depth of contamination: Small plants like ferns and grasses have been used where contamination is shallow. Because tree roots grow deeper, trees such as poplars and willows are used for hydraulic control or to clean up deeper soil contamination and contaminated groundwater.
4. Plant growth and development stage. Phytoremediation is most effective during the vegetative growth stages of plants. Plant vegetative growth stage is the most important phase for phytoremediation (Nie et al. 2011).
5. Type and properties of inoculum used for bioaugmentation.
6. Soil condition: Soil abiotic and biotic factors may determine the survival and activity of the introduced microorganisms (Juhanson et al. 2007). Some of the abiotic factors include temperature, soil pH, soil organic matter, soil moisture, cation exchange capacity, etc.
7. Bioavailability of contaminant to the microbial community is another factor influencing biodegradation of pollutants.
8. Age of the contaminants.
9. Physical and chemical properties of the contaminant. Contaminants that are soluble in water may pass by the root system without being accumulated.
10. Climatic factors. Plant survival and growth are adversely affected by extreme climatic factors.
11. Toxicity of soil.
12. Bioavailability of contaminant to plants. Metal that is tightly bound to the organic portions of the soil may not be available to plants.
13. Contaminant source.

15.6 Phytoremediation Strategies

These technologies to be discussed below are based on the plant's ability to absorb, accumulate, sequester and detoxify toxic metals:

1. *Hydraulic control* : In this process of phytoremediation, plants act like a pump, drawing the groundwater up through their roots to keep it from moving. It reduces the movement of contaminated groundwater towards clean areas off-site.
2. *Phytoaccumulation (phytoextraction)* : Plants absorb, accumulate and transport pollutants from the soil to aboveground plant parts (shoots). Removing the metals is as simple as pruning or cutting the plant aboveground mass. Plants, and their associated soil microbes, can release chemicals that act as biosurfactants in the soil that increase the uptake of contaminants. The aboveground plant parts rich in accumulated metal can be easily and safely processed by drying, ashing or composting. The plants used in a phytoextraction scheme should ideally have large biomass production and accumulate high concentration of metals in the aboveground portions (Adesodun et al. [2010](#)). Over 500 plant species (101 families) and approximately 0.2 % of angiosperms have been reported to possess metal hyperaccumulation ability (Krämer [2010](#)).
3. *Phytostabilization* involves the use of plants to reduce the mobility and bioavailability of contaminants in soil either through precipitation or adsorption onto roots. Plants adsorb contaminants onto their roots where microorganisms that live in the soil break down the adsorbed contaminants to less harmful chemicals. Mycorrhizal association, for example, is known to inhibit transport of metallic cations into plant roots. Some plant species such as *Combretum* and *Rhus* (Anacardiaceae) have the ability of in situ stabilization of some metals (Regnier et al. [2009](#) ; Mokgalaka-Matlala et al. [2010](#)).
4. *Phytodegradation* is the breaking down of contaminants into less toxic substances in the soil through the activities of microorganisms in the rhizosphere of plant roots or externally through metabolites produced by plants. For instance, exudates (peptides) from the bacterium *Pseudomonas putida* can decrease cadmium (Cd) toxicity in plants. Natural exudates such as siderophores, organic acids and phenolics released by the roots of certain plants can form complexes (chelates) with metals in the rhizosphere.

Table 15.1 Phytoremediation strategies of various groups of contaminants

Technology	Action on contaminants	Main type of contaminant	Vegetation
Phytostabilization	Retained in situ	Organics and metals	Cover maintained
Phytodegradation	Attenuated in situ	Organics	Cover maintained
Phytovolatilization	Removed	Organics and metals	Cover maintained
Phytoextraction	Removed	Metals	Harvested repeatedly
Phytofiltration	Retained in situ	Metals	Cover maintained

Metals such as the toxic Cr(III) can be converted to the much less toxic Cr(VI) by enzymes found on the roots of wetland plants. During detoxification plants release glutathione conjugates into the rhizosphere where they could be metabolized by microbes (Schroder et al. 2007).

5. **Phytovolatilization** involves use of plants to take up certain contaminants and then converts them into gaseous forms that vaporize into the atmosphere. This process is driven by the evapotranspiration of plants. Plants that have high evapotranspiration rate are sought after in phytovolatilization. Organic contaminants, especially volatile organic compounds (VOCs), are passively volatilized by plants. For example, hybrid poplar trees have been used to volatilize trichloroethylene (TCE) by converting it to chlorinated acetates and CO₂. Metals such as Se can be volatilized by plants through conversion into dimethylselenide [Se (CH₃)₂]. Genetic engineering has been used to allow plants to volatilize specific contaminants. For example, the ability of the tulip tree (*Liriodendron tulipifera*) to volatilize methyl-Hg from the soil into the atmosphere (as HgO) was improved by inserting genes of modified *E. coli* that encode the enzyme mercuric ion reductase.
6. **Phytofiltration:** This involves rhizofiltration where contaminants such as metals are precipitated within the rhizosphere. Metal plaque forms typically on the roots of wetland plants through the release of oxygen via the parenchyma of roots. Iron oxides, for example, can precipitate along with other metals into the metal plaque. Metal plaque on roots acts as a reservoir for active iron (Fe²⁺), which in turn increases the tolerance of plants to other toxic metals (Table 15.1).

The ability of the plants to degrade or metabolize xenobiotic pollutants can be improved by transferring genes from organisms (bacteria, fungi, plant and animals) which have potential for degradation and mineralization of xenobiotic pollutants. That is, catabolic genes essential for the degradation of contaminants are boosted in a plant resulting in enhanced phytoremediation. The plants which received the genes are called transgenic plants. The genes are introduced into the candidate plants using *Agrobacterium*-mediated or direct DNA method of gene transfer. Phytoremediation process in plant can also be improved by constructing plants with enhanced secretion of enzymes capable of degrading xenobiotics into the rhizosphere (Gerhardt et al. 2009) (Tables 15.2 and 15.3).

Table 15.2 General processes affecting rhizoremediation

Processes	Effects
Root exudates	Microbial growth stimulation
O ₂ —redox reaction	Microbial growth stimulation
CO ₂ —soil pH plant chelators and biosurfactants	Contaminant bioavailability
H ⁺ & OH ⁻ —soil pH acid/base reaction	Contaminant bioavailability
Microbial enzymes	Plant growth
Ion uptake	Plant growth
Microbial chelator—plant nutrient delivery	Plant growth

15.7 Phytoremediators

PHYTOREM database (compiled by Environment Canada) estimates that more than 750 plant species worldwide have potential for phytoremediation (Sarma 2011). Some of these include:

Bromus hordeaceus, *Festuca arundinacea*, *Trifolium fragiferum*, *Trifolium hirtum*, *Vulpia microstachys*, *Bromus carinatus*, *Elymus glaucus*, *Festuca rubra*, *Hordeum californicum*, *Leymus triticoides*, *Nassella pulchra*, *Combretum* sp., *Rhus* sp., *Phragmites australis*, *Alyssum corsicum*, *Alyssum murale*, mustard greens, *Helianthus annuus*, *Agrostis tenuis*, *Thlaspi caerulescens* (alpine pennycress), *Brassica juncea* (Indian mustard), *Liriodendron tulipifera* (yellow poplar) and *Nicotiana glauca* (Table 15.4).

15.8 Phytoremediation Traits

Plant adaptation and responses to contaminated environments depend on many physiological, molecular, genetic and ecological traits. Indicators of a plant's phytoremediation potential include the following:

1. Tolerance to high pH and salinity.
2. Tolerance to extreme drought and waterlogged conditions.
3. Good rooting system and adequate depth of root zone. Fibrous rooting system provides large surface area for root-soil contact.
4. High level of tolerance with respect to the contaminant known to exist at the site.
5. High growth rate and biomass yield.

Table 15.3 Some examples of microorganisms used for phytoremediation

Plants	Microbes	Contaminants	References
Sugar beets	<i>Pseudomonas</i> sp.	PCBs	Villacieros et al. 2005
<i>Thlaspi goesingense</i>	<i>Methylobacterium</i> sp.	Nickel	Idris et al. 2004
Rock cress	<i>Pseudomonas</i> sp.	PCBs	Narasimhan et al. 2003
Alfalfa	<i>Pseudomonas</i> sp.	PCBs	Brazil et al. 1995
Wheat	<i>Pseudomonas</i> sp.	TCE	Yee et al. 1998
<i>Thlaspi goesingense</i>	<i>Sphingomonas</i> sp.	Nickel	Idris et al. 2004
Wild rye	<i>Pseudomonas</i> sp.	Chlorobenzoic acid	Siciliano and Germida 1998
Pea	<i>Pseudomonas</i> sp.	24-D	Germaine et al. 2006
Poplar	<i>Pseudomonas</i> sp.	MTBE, TCE, BTEX	Germaine et al. 2004; Moore et al. 2006
Pea	<i>Pseudomonas</i> sp.	Naphthalene	Germaine et al. 2009
Barmultra grass	<i>Pseudomonas</i> sp.	Naphthalene	Kuiper et al. 2004
Barley	<i>Pseudomonas</i> sp.	Phenanthrene	Anokhina et al. 2004
Common reed	<i>Sinorhizobium meliloti</i> P221	Phenanthrene	Golubev et al. 2009
Switch grass	Indigenous degraders	PCBs	Chekol et al. 2004
Red clover, ryegrass	Indigenous degraders	24-D	Shaw and Burns 2004
Ryegrass	Indigenous degraders	PCPs	He et al. 2005
White mustard	Indigenous degraders	Petroleum hydrocarbon	Liste and Prutz 2006
Hybrid poplar	Indigenous degraders	BTEX, toluene	Barac et al. 2009
English oak, common ash	Indigenous degraders	TCE, toluene	Weyens et al. 2009
Birch	Indigenous degraders	PAHs	Sipilä et al. 2008
Altai wild rye, tall wheat grass	Indigenous degraders	Petroleum hydrocarbon	Phillips et al. 2009
Corn	<i>Gordonia</i> sp. S2Rp-17	Diesel	Hong et al. 2011
Yellow lupine	<i>Burkholderia cepacia</i>	Toluene	Barac et al. 2009
Poplar	<i>Burkholderia cepacia</i>	Toluene	Taghavi et al. 2005
Barley	<i>Burkholderia cepacia</i>	24-D	Jacobsen, 1997
Wheat	<i>Azospirillum lipoferum</i> spp.	Crude oil	Muratova et al. 2005; Shaw and Burns 2004
Tall fescue grass	<i>Azospirillum brasilense</i> Cd	PAHs	Huang et al. 2004
Tall fescue grass	<i>Enterobacter cloacae</i> CAL2	PAHs	Huang et al. 2004
Poplar	<i>Methylobacterium populi</i> BJ001	TNT, RDX, HMX	Van Aken et al. 2004a; Van Aken et al. 2004b

PAH polycyclic aromatic hydrocarbon; TCE trichloroethylene; PCB polychlorinated biphenyl; MTBE methyl tert-butyl ether; BTEX benzene, toluene, ethylbenzene and xylenes; 24-D 2,4-dichlorophenoxyacetic acid; PCP pentachlorophenol; TNT 2,4,6-trinitrotoluene; RDX hexahydro-1,3,5-trinitro-1,3,5-triazine; HMX octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine

Table 15.4 Some examples of phytoremediators and contaminants

Plants	Metals	References
<i>Arabis gemmifera</i>	Cd and Zn	Kubota and Takenaka 2003
<i>Crotalaria dactylon</i>	Ni and Cr	Saraswat and Rai 2009
<i>Thlaspi caerulescens</i>	Cd and Zn	Kupper and Kochian 2010
<i>Pelargonium</i> sp.	Cd	Dan et al. 2002
<i>Arabidopsis halleri</i>	Cd	Kupper et al. 2000
<i>Crotalaria juncea</i>	Ni and Cr	Saraswat and Rai 2009
<i>Thlaspi caerulescens</i>	Cd, Pb and Zn	Banasova and Horak 2008
<i>Brassica napus</i>	Cd	Selvam and Wong 2009
<i>Arabidopsis thaliana</i>	Zn and Cd	Saraswat and Rai 2009
<i>Thlaspi caerulescens</i>	Zn, Cd and Ni	Assuncao and Schat 2003
<i>Pistia stratiotes</i>	Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn	Odjegba and Fasidi 2004
<i>Chengiopanax sciadophylloides</i>	Mn	Mizuno et al. 2008
<i>Pteris vittata</i>	As	Dong 2005
<i>Sedum alfredii</i>	Pb and Zn	Sun et al. 2005
<i>Tamarix smyrnensis</i>	Cd	Manousaki et al. 2008
<i>Potentilla griffithii</i>	Zn and Cd	Hu et al. 2009
<i>Rorippa globosa</i>	Cd	Sun et al. 2010

6. High level of tolerance to waterlogging and extreme drought condition.
7. High level of accumulation, translocation and uptake potential of contaminant.
8. Habitat preference of plant, e.g. terrestrial aquatic or semiaquatic.

15.9 Effects of the Metals on the Phytoremediators

Plants that have been successfully used as phytoremediators were able to tolerate, accumulate or translocate the metals by reasons of the following effects of the metals on the plants:

1. The plant physiology: Metals affect the physiology of plants either by promoting or inhibiting the growth of the plant. Some develop metal tolerance characteristics through apoplastic or symplastic detoxification mechanisms (Pilon-Smits et al. 2009). Some are absorbed from soil solution through passive transport. Hg, for example, may preferentially bind with sulphur- and nitrogen-rich ligands (amino acids) and enter inside the cells. Cd can induce changes in lipid profile (Ouariti et al. 1997) and can also affect the enzymatic activities associated with membranes such as the H⁺ATPase (Fodor et al. 1995).
2. Biomass production of the plants that have been successfully used as phytoremediators.

15.10 Responses of Microbial Communities to Phytoremediation

Different plant species have different effects on microorganisms in the soil. For instance, *Alyssum corsicum*, *Alyssum murale* and *Brassica juncea* (Ni hyperaccumulators) have been reported to increase both the population and biomass of soil microorganisms. By absorbing nickel from the soil and excreting root exudates, the plants reduced nickel toxicity and improved the living environment of the microbes (Cai et al. 2007). Phytoremediation increased the number of phenol-degrading bacteria as well as metabolic diversity of microbial community in semi-coke polluted soil (Truu et al. 2003). Perennial ryegrass supports a general increase in microbial activity and numbers in the rhizosphere, some of which have catabolic activity towards petroleum hydrocarbons in petroleum-contaminated soil. Alfalfa, on the other hand, seems to specifically increase the number of microorganisms capable of degrading more complex hydrocarbons (Kirk et al. 2005). Plant-dependent changes in microbial functionality are the result of some form of communication between the associated microorganisms and the plant. For example, bacterial products, such as lumichrome, stimulate root respiration and thereby increase the availability of root exudates for bacteria (Phillips et al. 2009).

15.11 Sources of Environmental Pollution

1. Increased toxic waste from increased population
2. Anthropogenic activities such as agriculture
3. Metal purification procedure, which includes mining, smelting and the tailings from industries

References

- Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA (2010) Phytoremediation potentials of Sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with Zinc and Lead nitrates. Water Air Soil Pollut 207:195–201. doi: [10.1007/s11270-009-0128-3](https://doi.org/10.1007/s11270-009-0128-3)
- Anokhina TO, Kochetkov VV, Zelenkova NF, Balakshina VV, Boronin AM (2004) Biodegradation of phenanthrene by *Pseudomonas* bacteria bearing rhizospheric plasmids in model plant: microbial associations. Appl Biochem Microbiol 40:568–572. doi: [10.1023/B:ABIM.0000046992.01220.35](https://doi.org/10.1023/B:ABIM.0000046992.01220.35)
- Assuncao AGL, Schat H (2003) *Thlaspi caerulescens* an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159:351–360. doi: [10.1046/j.1469-8137.20000820.x](https://doi.org/10.1046/j.1469-8137.20000820.x)

Banasova V, Horak O (2008) Heavy metal content in *Thlaspi caerulescens* J. et C. presl growing on metalliferous and non-metalliferous soils in Central Slovakia. *Int J Environ Pollut* 33:133–145. doi: [10.1504/IJEP.2008.019388](https://doi.org/10.1504/IJEP.2008.019388)

Barac T, Weyens N, Oeyen L, Taghavi S, van der Lelie D, Dubin D, Spliet M, Vangronsveld J (2009) Field note: hydraulic containment of BTEX plume using poplar trees. *Int J Phytoremediation* 11:416–424. doi: [10.1080/15226510802655880](https://doi.org/10.1080/15226510802655880)

Brazil GM, Kenefick L, Callanan M, Haro A, de Lorenzo V, Dowling DN, O'Gara F (1995) Construction of a rhizosphere pseudomonad with potential to degrade polychlorinated biphenyls and detection of bph gene expression in the rhizosphere. *Appl Environ Microbiol* 61:1946–1952. aem.asm.org/content/61/5/1946

Cai X, Qiu R, Chen G, Zeng X, Fang X (2007) Response of microbial communities to phytoremediation of nickel contaminated soils. *Front Agric China* 1:289–295. doi: [10.1007/s11703-007-0049-0](https://doi.org/10.1007/s11703-007-0049-0)

Chekol T, Vough LR, Chaney RL (2004) Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect. *Environ Int* 30:799–804. doi: [10.1016/j.envint.2004.01.008](https://doi.org/10.1016/j.envint.2004.01.008)

Dan TV, Krishnaraj S, Saxena PK (2002) Cadmium and nickel uptake and accumulation in scented geranium (*Pelargonium* sp. Frensham). *Water Air Soil Pollut* 137:355–364. doi: [10.1023/A:1015590007901](https://doi.org/10.1023/A:1015590007901)

Danh LT, Truong P, Mammucari R, Tran T, Foster N (2009) Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. *Int J Phytorem* 11:664–691. doi: [10.1080/15226510902787302](https://doi.org/10.1080/15226510902787302)

Dong R (2005) Molecular cloning and characterization of a phytochelatin synthase genes, PvPCS1 from *Pteris vittata* L. *J Ind Microbiol Biotechnol* 32:527–533. doi: [10.1007/s10295-005-0234-1](https://doi.org/10.1007/s10295-005-0234-1)

Fodor E, Szabo-Nagy A, Erdei L (1995) The effects of cadmium on the fluidity and H⁺ ATPase activity of plasma membrane from sunflower and wheat roots. *J Plant Physiol* 147:87–92. doi: [10.1016/S0176-1617](https://doi.org/10.1016/S0176-1617)

Gerhardt KE, Huang XD, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Sci* 176:20–30. doi: [10.1016/j.plantsci.2008.09.014](https://doi.org/10.1016/j.plantsci.2008.09.014)

Germaine K, Keogh E, Cabellos GG, Borremans B, van der Lelie D, Barac T, Oeyen L, Vangronsveld J, Moore FP, Moore ERB, Campbell CD, Ryan D, Dowling DN (2004) Colonisation of poplar E.T. Alori
aloritope@yahoo.com

189

trees by *gfp* expressing bacterial endophytes. *FEMS Microbiol Ecol* 48:109–118. doi: [10.1016/j.femsec.20012.009](https://doi.org/10.1016/j.femsec.20012.009)

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. *FEMS Microbiol Ecol* 57:302–310. doi: [10.1111/j.1574-6941.2006.00121.x](https://doi.org/10.1111/j.1574-6941.2006.00121.x)

Germaine KJ, Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. *FEMS Microbiol Lett* 296:226–234. doi: [10.1111/j.1574-6968.2009.01637.x](https://doi.org/10.1111/j.1574-6968.2009.01637.x)

Golubev S, Schelud'ko A, Muratova A, Makarov O, Turkovskaya O (2009) Assessing the potential of rhizobacteria to survive under Phenanthrene pollution. *Water Air Soil Pollut* 198:5–16. doi: [10.1007/s11270-008-9821-x](https://doi.org/10.1007/s11270-008-9821-x)

Haque N, Peralta-Videa JR, Jones GL, Gill TE, Gardea-Torresdey JL (2008) Screening the phytoremediation potential of desert broom (*Baccharis sarothroides* Gray) growing on mine tailings in Arizona, USA. *Environ Pollut* 153:362–368. doi: [10.1016/j.envpol.2007.08.024](https://doi.org/10.1016/j.envpol.2007.08.024)

He Y, Xu JM, Tang CX, Wu YP (2005) Facilitation of pentachlorophenol degradation in the rhizosphere of ryegrass (*Lolium perenne* L). *Soil Biol Biochem* 37:2017–2024. doi: [10.1016/j.soilbio.2005.0002](https://doi.org/10.1016/j.soilbio.2005.0002)

Hong S, Kim D, Baek S, Kwon S, Samson RA (2011) Taxonomy of Eurotium species isolated from meju. *J Microbiol* 49(4):669–674. doi: [10.1007/s12275-011-0376-y](https://doi.org/10.1007/s12275-011-0376-y)

Hu PJ, Qiu RL, Senthilkumar P, Jiang D, Chen ZW, Tang YT, Liu FJ (2009) Tolerance accumulation and distribution of zinc and cadmium in hyperaccumulator *Potentilla griffithii*. *Environ Exp Bot*

66:317–325. doi: [10.1016/j.envexpbot.2009.02.014](https://doi.org/10.1016/j.envexpbot.2009.02.014)

Huang X, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004) A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. *Environ Pollut* 130:465–476. doi: [10.1016/j.envpol.20009.031](https://doi.org/10.1016/j.envpol.20009.031)

Idris A, Inane B, Hassan MN (2004) Overview of waste disposal and landfills/dumps in Asian countries. *J Mater Cy Waste Manag* 6:104–110. doi: [10.1007/s10163-004-0117-y](https://doi.org/10.1007/s10163-004-0117-y)

Jacobsen CS (1997) Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia (pseudomonas) cepacia* DBOL (pRO101) in 2, 4-D contaminated soil. *Plant Soil* 189:139–144. doi: [10.1023/A:1004296615446](https://doi.org/10.1023/A:1004296615446)

Juhanson J, Truu J, Heinaru E, Heinaru A (2007) Temporal dynamics of microbial community in soil during phytoremediation field experiment. *J Environ Engineering Lands Manag* 14:213–220. doi: [10.1080/16486897.2007.9636933](https://doi.org/10.1080/16486897.2007.9636933)

Kirk JL, Klironomos JN, Lee H, Trevors JT (2005) The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum-contaminated soil. *Environ Pollut* 133:455–465. doi: [10.1016/j.envpol.2004.06.002](https://doi.org/10.1016/j.envpol.2004.06.002)

Krämer U (2010) Metal hyperaccumulation in plants. *Annu Rev Plant Biol* 61:517–534. doi: [10.1146/annurev-arplant-042809-112156](https://doi.org/10.1146/annurev-arplant-042809-112156)

Kubota H, Takenaka C (2003) *Arabidopsis gemmifera* is a hyperaccumulator of Cd and Zn. *Int J Phytorem* 5:197–220. doi: [10.1080/713779219](https://doi.org/10.1080/713779219)

Kuiper I, Bloemberg GV, Lugtenberg BJJ (2001) Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. *Mol Plant Microbe Interact* 14:1197–1205. doi: [10.1094/MPMI.2001.14.10.1197](https://doi.org/10.1094/MPMI.2001.14.10.1197)

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. *Mol Plant Microbe Interact* 17:6–15. doi: [10.1094/MPMI.2004.17.1.6](https://doi.org/10.1094/MPMI.2004.17.1.6)

Kupper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta* 212:75–84. doi: [10.1007/s004250000366](https://doi.org/10.1007/s004250000366)

Kupper H, Kochian LV (2010) Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* (Ganges population). *New Phytol* 185:114–129. doi: [10.1111/j.1469-8137.2009.03051.x](https://doi.org/10.1111/j.1469-8137.2009.03051.x)

Liste HH, Prutz I (2006) Plant performance, Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* (Ganges population) bacteria, and biodegradation of weathered hydrocarbons in contaminated soil. *Chemosphere* 62:1411–1420. doi: [10.1016/j.chemosphere.2005.05.018](https://doi.org/10.1016/j.chemosphere.2005.05.018)

Manousaki E, Kadukova J, Papadantonakis N, Kalogerakis N (2008) Phytoextraction and phytoremediation of Cd by the leaves of *Tamarix smyrnensis* growing on contaminated non saline and saline soils. *Environ Res* 106:326–332. doi: [10.1016/j.jenvres.2007.04.004](https://doi.org/10.1016/j.jenvres.2007.04.004)

Mizuno T, Hirano K, Kato S, Obata H (2008) Cloning of ZIP family metal transporter genes from the manganese hyperaccumulator plant *Chenopodium sciadophylloides* and its metal transport and resistance abilities in yeast. *Soil Sci Plant Nutr* 54:86–94. doi: [10.1111/j.1747-0765.2007.00206.x](https://doi.org/10.1111/j.1747-0765.2007.00206.x)

Mokgalaka-Matlala NS, Regnier TC, Combrinck S, Weiersbye IM (2010) Selection of tree species as assets for mine Phytoremediation using the genus *Rhus* (Anacardiaceae) as a model. In: Fourie AB, Tibbett M (eds) Proceedings of the 5th International Conference on Mine Closure 2010, Australian Centre for Geomechanics, Perth, Australia, p 343–350

Moore FP, Barac T, Borrenans B, Oeyen L, Vangronsveld J, van der Lie D, Cambell CD, Moore ERB (2006) Endophytic bacterial diversity in poplar trees growing on BTEX contaminated site: the characterisation of isolates with potential to enhance phytoremediation. *Syst Appl Microbiol* 29:539–556. doi: [10.1016/j.syapm.2005.11.012](https://doi.org/10.1016/j.syapm.2005.11.012)

Muratova AY, Turkovskaya OV, Antonyuk LP, Makarov OE, Pozdnyakova LI, Ignatov VV (2005) Oil-oxidizing potential of associative rhizobacteria of the genus *Azospirillum*. *Microbiology* 74:210–215. doi: [10.1007/s11021-005-0053-4](https://doi.org/10.1007/s11021-005-0053-4)

Narasimhan K, Basheer C, Bajic VB, Swarup S (2003) Enhancement of

plant-microbe interactions using a rhizosphere metabolomics-driven approach and its application in the removal of polychlorinated biphenyls. *Plant Physiol* 132:146–153. doi: [10.1104/pp.102.016295](https://doi.org/10.1104/pp.102.016295)

Nie M, Zhang XD, Wang JQ, Jiang LF, Yang J et al (2009) Rhizosphere effects on soil bacterial abundance and diversity in the Yellow River Deltaic ecosystem as influenced by petroleum contamination and soil salinization. *Soil Biol Biochem* 41:2535–2542. doi: [10.1016/j.soilbio.2009.09.012](https://doi.org/10.1016/j.soilbio.2009.09.012)

Nie M, Wang Y, Yu J, Xiao M, Jiang L et al (2011) Understanding plant-microbe interactions for phytoremediation of petroleum-polluted soil. *PLoS One* 6:e17961. doi: [10.1371/journal.pone.0017961](https://doi.org/10.1371/journal.pone.0017961)

Odjegba VJ, Fasidi IO (2004) Accumulation of trace elements by *Pistia stratiotes*: implications for phytoremediation. *Ecotoxicology* 13:637–646. doi: [10.1007/s10646-003-4424-1](https://doi.org/10.1007/s10646-003-4424-1)

Ouariti O, Boussama N, Zarrouk M, Cherif A, Ghorbali MH (1997) Cadmium and copper induced changes in tomato membranes lipids. *Phytochemistry* 45:1343–1350. doi: [10.1016/S0031-9422\(97\)00159-3](https://doi.org/10.1016/S0031-9422(97)00159-3)

Phillips LA, Greer CW, Farrell RE, Germida JJ (2009) Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatment. *Appl Soil Ecol* 42(1):9–17. doi: [10.1016/j.apsoil.2009.01.002](https://doi.org/10.1016/j.apsoil.2009.01.002)

Pilon-Smiths EAH, Quinn CF, Tapken W, Malagoni M, Schiavon M (2009) Physiological functions of beneficial elements. *Curr Opin Plant Biol* 12:267–274. doi: [10.1016/j.pbi.2009.04.009](https://doi.org/10.1016/j.pbi.2009.04.009)

Regnier TC, Kouekam CR, Leonard CM, Combrink S, Mokgalaka NS, Weiersbye IM (2009) Chemical analysis and potential use of the tree *Combretum erythrophyllum* grown on gold and uranium mine tailings seepage. In: Fourie A, Tibbett M (eds) *Proceedings of the 4th International Conference on Mine Closure*, Perth, Australia, p 539–547

15 Phytoremediation Using Microbial Communities: II

aloritope@yahoo.com

190

Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *J Environ Sci Technol* 4:118–138. doi: [10.3923/jest.2011.118.138](https://doi.org/10.3923/jest.2011.118.138)

Saraswat S, Rai JPN (2009) Phytoextraction potential of six plant species grown in multimetal contaminated soil. *Chem Ecol* 25:1–11. doi: [10.1080/02757540802657185](https://doi.org/10.1080/02757540802657185)

Schroder P, Scheer CE, Diekmann F, Stampfl A (2007) How plants cope with foreign compounds: translocation of xenobiotic glutathione conjugates in roots of barley (*Hordeum vulgare*). *Environ Sci Pollut Res Int* 14:114–122

Selvam A, Wong JWC (2009) Cadmium uptake potential of *Brassica napus* cocropped with *Brassica parachinensis* and *Zea mays*. *J Hazard Mater* 167:170–178. doi: [10.1016/j.jhazmat.2008.12.103](https://doi.org/10.1016/j.jhazmat.2008.12.103)

Shaw LJ, Burns RG (2004) Enhanced mineralization of [U-C-14]2,4-dichlorophenoxyacetic acid in soil from the rhizosphere of *Trifolium pratense*. *Appl Environ Microbiol* 70:4766–4774. doi: [10.1128/AEM.70.8.4766-4774.2004](https://doi.org/10.1128/AEM.70.8.4766-4774.2004)

Siciliano SD, Germida JJ (1998) Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. *Environ Rev* 6:65–79. doi: [10.1139/a98-005](https://doi.org/10.1139/a98-005)

Siciliano SD, Germida JJ, Banks K, Greer CW (2003) Changes in microbial community composition and function during a polycyclic aromatic hydrocarbon phytoremediation field trial. *Appl Environ Microbiol* 69:483–489. doi: [10.1128/AEM.69.483-489.2003](https://doi.org/10.1128/AEM.69.483-489.2003)

Sipilä TP, Keskinen AK, Åkerman ML, Fortelius C, Haahtela K, Yrjölä K (2008) High aromatic ring-cleavage diversity in birch rhizosphere: PAH treatment-specific changes of I.E.3 group extradiol dioxygenases and 16S rRNA bacterial communities in soil. *ISME J* 2:968–981. doi: [10.1038/ismej.2008.50](https://doi.org/10.1038/ismej.2008.50)

Sun Q, Ye ZH, Wang XR, Wong MH (2005) Increase of glutathione in mine population of *Sedum alfredii*: a Zn hyperaccumulator and Pb accumulator. *Phytochemistry* 66:2549–2556. doi: [10.1016/j.phytochem.2005.08.012](https://doi.org/10.1016/j.phytochem.2005.08.012)

Sun R, Jin C, Zhou Q (2010) Characteristics of cadmium accumulation and tolerance in *Rorippa globosa* (Turez) Thell., a species with some characteristics of cadmium hyperaccumulation. *Plant Growth*

Regul 61:67–74. doi: [10.1007/s10725-010-9451-3](https://doi.org/10.1007/s10725-010-9451-3)

Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J, van der Lelie D (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene.

Appl Environ Microbiol 71:8500–8505. doi: [10.1128/AEM.](https://doi.org/10.1128/AEM.71.12.8500-8505.2005)

[71.12.8500-8505.2005](https://doi.org/10.1128/AEM.71.12.8500-8505.2005)

Truu J, Talpsep E, Velder E, Heinaru E, Heinaru A (2003) Enhanced biodegradation of oil-shale chemical industry solid wastes by phytoremediation and bioaugmentation. Oil Shale 20:421–428

Van Aken B, Peres CM, Doty SL, Yoon JM, Schnoor JL (2004a)

Methylobacterium populi sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (*Populus deltoides* x *nigra* DN34). Int J Syst Evol Microbiol 54:1191–1196. doi: [10.1099/ijs.0.02796-0](https://doi.org/10.1099/ijs.0.02796-0)

Van Aken B, Yoon JM, Schnoor JL (2004b) Biodegradation of nitrosubstituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-

1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic *Methylobacterium* sp. associated with poplar tissues (*Populus deltoides* x *nigra* DN34). Appl Environ Microbiol

70:505–517

Villacieros M, Whelan C, Mackova M, Molgaard J, Sánchez-Contreras M, Lloret J, Aguirre de Cárcer D, Oruezábal RI, Bolaños L, Macek T,

Karlson U, Dowling DN, Martín M, Rivilla R (2005) Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 derivatives,

using a *Sinorhizobium meliloti* nod system to drive bph gene

expression. Appl Environ microbiol 71:2687–2694

Wenzel WW (2009) Rhizosphere processes and management in plantassisted bioremediation (phytoremediation) of soils. Plant Soil

321:385–408. doi: [10.1007/s11104-008-9686-1](https://doi.org/10.1007/s11104-008-9686-1)

Weyens N, Taghavi S, Barac T, van der Lelie D, Boulet J, Artois T, Carleer R, Vangronsveld J (2009) Bacteria associated with oak and

ash on a TCE-contaminated site: characterization of isolates with potential to avoid evapotranspiration of TCE. Environ Sci Pollut

Res 16:830–843. doi: [10.1007/s11356-009-0154-0](https://doi.org/10.1007/s11356-009-0154-0)

Yee DC, Maynard JA, Wood TK (1998) Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonas fluorescens*

strain expressing toluene ortho-monoxygenase constitutively.

Appl Environ Microbiol 64:112–118

E.T. Alori

aloritope@yahoo.com